

The University of Chicago

**EMBRYONIC DEVELOPMENT OF THE
RESPIRATORY PORTION OF
THE PIG'S LUNG**

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ANATOMY

1935

By
LEO P. CLEMENTS

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EMBRYONIC DEVELOPMENT OF THE RESPIRATORY PORTION OF THE PIG'S LUNG¹

LEO P. CLEMENTS

Department of Anatomy, The University of Chicago and Creighton Medical School

THREE PLATES (SIX FIGURES)

The question of the presence or absence of a histological epithelium lining the respiratory portion of the mammalian lung cannot be settled until the changes which occur during the transition from late fetal to postnatal stages are thoroughly studied. It is not necessary to review here the controversial literature on the embryonic and postnatal mammalian lung as this has been adequately treated in the works of Fried ('34), Bremer ('35) and Bargmann ('36). The present study, based on close, successive stages of very fresh fetal and postnatal pig lungs, deals with the formation of the respiratory spaces and the fate of the connective tissue and the epithelium of the terminals of the bronchial tree. Particular attention has been paid to the arrangement of the blood capillaries in the developing respiratory portion of the lung.

MATERIALS AND METHODS

Fetal pigs from sixty-one litters were secured at Cudahy's abattoir, Omaha, so that the 130 fetal and three postnatal pigs prepared represented thirty fetal stages based on millimeter crown-rump length, and two postnatal stages, as follows (number of litters in parentheses): (1) 55, (1) 70, (2) 75, (1) 80, (1) 90, (1) 115, (2) 120, (1) 130, (2) 140, (3) 155, (1) 165, (5) 170, (1) 175, (1) 180, (2) 185, (1) 190,

¹ This investigation was aided by a grant of the Rockefeller Foundation to The University of Chicago.

(2) 195, (5) 200, (1) 210, (1) 215, (4) 220, (4) 230, (1) 235, (4) 240, (1) 250, (3) 255, (2) 260, (1) 270, (1) 275, and (3) 290 mm., the fetal length at term. One postnatal specimen was six and two (one litter) were 18 hours old. Stages 200 to 240 mm. proved critical in development of the respiratory portion.

The fetal lungs were fixed in Zenker-formol within 5 minutes after removal of the uterus from the sow, and about 30 minutes after the sow was killed. In many cases the fetal heart was beating vigorously when exposed. The lungs, trachea, heart and larger blood vessels were always removed and placed in the fixative together to avoid mechanical injury.

Where there were four pigs in a litter, in one the lung was sliced at 1-cm. intervals with a thin razor blade before it was removed from the chest cavity. In a second the fixative was injected via the trachea at low pressure before the thoracic cavity was opened. In the third, when the heart was still beating the pulmonary veins were clamped and the heart allowed to contract several times to force more blood into the lung before removal. In the fourth, the lung was removed without previous treatment.

The tissues were embedded in nitrocellulose and sectioned at 13 μ . The sections were mounted in series, usually four sections to the slide and eight slides to the series, by the use of oil of cloves, after which the nitrocellulose was removed in ether-alcohol. To keep the sections uniform, the sections for a slide were lined up on the knife, well moistened with 75% alcohol, and then removed from the knife with thin paper and stored moist with 75% alcohol in Petri dishes until convenient to mount them. In mounting, each paper was placed, with the sections up, on a pad of smooth filter paper, and the albumenized slide pressed down on the sections so that they adhered to the slide when the paper was removed. After blotting to remove excess alcohol and to flatten the sections, clove oil was poured on them.

Heidenhain's iron hematoxylin, phosphotungstic acid hematoxylin, hematoxylin-eosin-azure II, Mallory-azan, Foot's

modification of Bielschowsky's silver impregnation, and orcein were used in each series. Both Mallory-azan and acridin red were used as counterstains after the Foot stain. Methyl green was used over the orcein.

In two of the 290-mm. and one 18-hour postnatal stage, 3% silver nitrate was injected by the trachea, and allowed to stand 15 to 20 minutes. Then the lung was immersed in 3% silver nitrate 3 hours, then in 10% formalin 24 hours, washed in water, dehydrated and embedded. After sectioning and mounting the silver was further reduced on the slide with Eastman Kodak Company D-73 developer, and the sections counterstained with Mallory-azan, hematoxylin-eosin-azure II, phosphotungstic acid hematoxylin and Harris' hematoxylin.

OBSERVATIONS

The pre-critical stages (55 mm., 115 mm. and 160 mm.)

At 55 mm. a section from a lung shows indefinite septa which divide the lobe into primordial lobules. The largest two or three orders of primordial tubules are lined with folded tall columnar epithelium. From these the remaining two or three orders branch and appear in each lobule as tubules with columnar epithelium and dilated ends, most of which branch dichotomously. The nuclei lie at the base of the epithelial cells or next to the lumen with equal frequency. Mitotic figures appear in all tubes, but more frequently in the smallest ones and their terminals. Cilia are not seen.

The connective tissue is condensed around the tubes and at their dilated ends where it is wider but less dense than along the sides. The wide spaces between the dense connective tissue are filled with loose connective tissue. The blood vessels and capillaries are more prominent in the loose connective tissue, but occasionally a capillary invades the dense connective tissue around the tubes. The reticular fibers form a loose network in the loose connective tissue and a more compact one in the denser tissue around the tubes, while at the base of the epithelium they form a very dense layer. At the terminals of the tubes the fibers of this layer are widely

scattered and arranged in roughly parallel semi-circles about the ends. As the growing terminal bud pushes into the connective tissue, these fibers form the condensed layer of reticular fibers at the base of the epithelium of the tubule.

At 115 mm. the number of primordial lobules has increased about fivefold as seen in sections without the aid of reconstruction. Around the largest tubes is a varying thickness of connective tissue continuous with that of the interlobular septa. The largest of these tubes are lined with folded pseudo-stratified columnar epithelium. The others, lined with tall columnar epithelium, give rise to four or five orders of mostly dichotomously branching tubes; a single section of a lobule presents twenty to thirty such tubules cut at various angles, and lined with columnar epithelium. The nuclei in the smaller tubules are chiefly at the apices of the cells. Mitoses occur in the epithelium as at 55 mm., and are seen often in the connective tissue cells. As the lung grows and the number of tubules increases, the area of loose connective tissue appears reduced in section so that now its width equals that of the dense tissue around the tubes. Cilia cannot be seen even in the largest tubes.

Capillaries are abundant in the connective tissue around the tubes and at their terminals where they occasionally extend through the condensed layer of reticular fibers to the base of the epithelium. Some capillaries even run between these fibers and the base of the epithelium.

At this stage, the reticular fibers show no change in distribution, although with the increase in the number of tubes they appear heavier. Some collagenous connective tissue and smooth muscle is being formed around the largest tubes and blood vessels, but around the smaller tubes the condensed layer of connective tissue contains only reticular fibers.

At 160 mm. a section from a lobe has roughly twice as many lobules as at 115 mm., but the lobules are smaller and the lobe, although larger, has not doubled in size. Each lobule in a given section contains two or three fairly large tubes lined with somewhat folded, tall columnar epithelium. From

these arise three or four orders of dichotomously branching smaller tubules lined with columnar epithelium. Some of the small tubules are lined with cuboidal epithelium. From the ends and sides of these tubes arise a large number of epithelial buds which appear as spherical, acinar masses of cells with little or no lumen connecting with that of the original tube, except at the periphery of the lobule where the buds are more open. In cross sections they appear as circles of cells in the dense connective tissue. Mitoses of epithelial cells are fairly frequent, especially in the small tubules. Cilia are seen in the large primitive bronchi, but not in any tubes in the lobules.

The increasing number of tubes and end buds are separated by dense connective tissue except for small stretches of loose connective tissue in the wider intervals. There are more collagenous fibers around the larger tubes and blood vessels, and in the interlobular septa.

The critical stage (200 to 220 mm.)

At 200 mm. the tubular complex in its progressive branching and growth into the connective tissue shows changes which forecast the definitive pulmonary structure of tubular air conductors and a respiratory portion. At this stage in development, the continuous epithelial lining of the tubules begins to become discontinuous and it accordingly might be called the critical stage. The epithelium in more of the largest tubes in the lobule appears pseudostratified (fig. 1, B) while the small tubules are lined with cuboidal epithelium, except in their end buds where it is columnar. Not far from the origin of the third or fourth order of tubes in the lobule, the epithelium may become cuboidal and later become discontinuous and leave the tubular wall bare of epithelium, except for occasional cells, isolated or in groups of two to four (fig. 2, I). The cuboidal and columnar cells in the end buds (figs. 1 to 5, EB), especially at the lobular periphery, persist long after the epithelium has disappeared from the stretches just proximal to them.

At 200 mm., a section from a lobe is considerably larger than at 160 mm. and shows a small increase in number of lobules, each of which contains three or four orders of tubes lined with pseudostratified or columnar epithelium, and a terminal order lined with cuboidal epithelium. The latter occasionally is discontinuous, especially around the angles where branches arise, so that a capillary or the underlying connective tissue is exposed to the lumen (figs. 1 and 2, EC). These tubules end with dividing epithelial buds, lined with columnar or cuboidal cells.

Mitoses are fairly frequent in the epithelium of the end buds. They appear less often than at 160 mm. in the lobular tubes, and gradually diminish toward the largest primordial bronchi, where they are few.

Practically all the connective tissue is dense between the tubules and their closely packed end buds. Capillaries are found more frequently than at 160 mm. among the reticular fibers at the base of the epithelium. There is the same relation of reticular fibers as at 160 mm. Collagenous connective tissue is more pronounced around the larger tubes and blood vessels.

Later stages (220 to 290 mm.)

At 220 to 230 mm. the number of lobules in a section of a lobe has increased somewhat over that at 200 mm. The lobules on the average are larger, and the lobe as a whole is considerably larger. In each lobule eight to twelve orders of branches are counted. The peripheral tubes are now irregularly elongated and expanded and have branches and sub-branches which end in wide sacs formed by the dividing end buds (fig. 2, S). Along the walls of these spaces proximal to the end buds much of the epithelium is absent, and capillaries and connective tissue are exposed to the lumen. As the distance increases between the ends of the epithelial lined tubules and the lobular boundary (figs. 1 to 5, B to P), the spaces between them become longer and assume somewhat the appearance of alveolar ducts as seen after birth. The epithelium is columnar to cuboidal in most of the end buds and

mitoses are fairly frequent; in some end buds the epithelium is incomplete. Mitoses are somewhat less frequent in the tubules than at 200 mm. and appear even less frequently in the largest primordial bronchi.

All of the connective tissue is now dense and is greatly reduced in amount between the enlarged, non-epithelial lined spaces and the great number of end buds at their terminals. These buds are 30 to 40 μ in diameter.

Along the walls of those spaces where the epithelium no longer forms a continuous membrane, the epithelial cells, singly or in small groups, are often shrunken and contain pycnotic nuclei. In places the lumen of such spaces is bounded by connective tissue cells partially incorporated in the condensed reticular fibers and the accompanying ground substance. Occasionally large flaky cells with small nuclei appear in the spaces; these are probably desquamated cells from the amnion or epidermis.

Capillaries are more frequent than at 200 mm. among the condensed reticular fibers lining the above described spaces and the end buds. Frequently they bulge into the lumen as loops, or run for short distances as naked capillaries along its wall. In these cases no membrane is seen between the red blood cells and the lumen except the endothelium with its nuclei. Where the capillaries touch the bases of epithelial cells, these separate and the capillaries become exposed to the future air passages. Occasionally a shrunken epithelial cell is seen over a capillary, or at the angle of its emergence through the reticular fibers. Where the continuous epithelium of a tubule ends a capillary often extends through the dense reticular fibers to be exposed as a loop in the future air space (fig. 3, EC'). In looking at these preparations, it is difficult to escape the conclusion that the capillaries are actively pushing through the connective tissue and the epithelium. In any event the sharp separation of epithelium from vascular connective tissue cells no longer holds.

The reticular fibers are increased in number and condensed at the base of the epithelium of the large primordial bronchi.

These fibers gradually decrease in the direction toward the end buds. With the increase in extent of the peripheral epithelium-free spaces and in the number of their end buds, the condensed reticular fibers of two adjacent spaces or of two end buds frequently become confluent and form a common support for the wall of the two spaces or buds. Reticular fibers are seldom seen over the exposed surface of a capillary at this stage, but occasionally are seen along its side. There are a few scattered collagenous fibers in the connective tissue about the finer ramifications of the tubules.

At 250 mm. the lobules are larger than at 220 to 230 mm., and their number is probably somewhat greater. In each lobule are four or five orders of tubules, the largest lined with folded tall columnar and the rest with columnar epithelium, except for a few cuboidal cells at the end of the epithelium of the tubule. From the latter out to the end buds there are now eight to ten orders of branches of epithelium-free spaces suggestive of alveolar ducts, which have widened and lengthened, so that the distance between the ends of the epithelium-lined tubules and the lobular periphery is further increased. The final branches of these 'spaces' end at the lobular periphery, and within the lobule, as expanded fan-shaped sacs formed by the dividing end buds which occupy their walls (fig. 3, S). Most of the end buds are opened as horseshoe-shaped cups, or spread out as wide crescents. A few remain as more or less solid buds. There is a marked thinning of the connective tissue walls between adjacent spaces. With the increase in size and number of the branching spaces the connective tissue is further decreased in amount and begins to appear lace-like. The epithelium of the peripheral spaces is limited chiefly to the end buds. At the lobular periphery the latter occupy much of the room along the connective tissue septum. Only short stretches of epithelial cells, and irregular isolated ones, are occasionally seen in the 'alveolar duct-like spaces.'

Capillaries are more prominent in the lining of the spaces and characteristically loop around the angle of evaginations from them. In many closely adjacent spaces, capillaries wind

through the thin wall to be exposed first to one lumen and then the other. There are more confluent strands of condensed reticular fibers in the thinner connective tissue walls between the duct-like spaces. There is no change in the collagenous fibers. Cilia appear in some large intralobular tubules.

At 275 mm. three or four orders of tubules lined with columnar epithelium are present in each section of a lobule. A few cuboidal cells line the ends of these tubules. The duct-like spaces devoid of a continuous epithelium have elongated greatly and form a close network in the future respiratory area, and the distance between the ends of the epithelium-lined tubules and the lobular periphery is greater than at 250 mm. End buds are now seldom seen except at the subpleural periphery where a few appear as irregular spaces lined in part with epithelial cells (fig. 4, EB). Nearly all of the 'ducts' next to the interlobular septa (not subpleural) are free of histological epithelium.

With the increase in size and number of the alveolar duct-like spaces, the connective tissue now appears as a network of thin strands among the future air spaces although some of the partitions have the thickness of three or four connective tissue cells; the lobule has an increasingly lace-like appearance.

Epithelial cells in the 'ducts' now appear only as occasional, isolated, apparently loosely attached, irregular cells with pycnotic nuclei. These spaces, which have elongated greatly, now look more like alveolar ducts of the postnatal animal, but the lack of alveoli in their walls, and the impression that they are still growing and branching preclude such a designation.

The walls between these 'ducts' contain endothelial cells, a few lymphocytes, and connective tissue cells. When the epithelium is absent, the latter frequently border on future air spaces. This is especially clearly shown in Foot silver preparations counterstained with Mallory-azan. In hematoxylin-eosin-azure II preparations a few of the connective tissue cells in the walls and at the angles of protruding capillaries, are larger than the average, are spherical to irregular

in outline, and their cytoplasm tends to be vacuolated and pinkish. Some of these are deep in the septal wall, but in others half of their bodies protrudes into the future air spaces. They are suggestively like the septal cells of Lang ('25).

There are increased numbers of capillaries exposed to the future air spaces in the walls of the 'ducts' and in their terminals where the epithelium is gone. With the thinning of the walls between adjacent spaces, capillaries wind through these walls exposed first to one potential air space and then another. In other places a septal wall lies between two capillaries, each exposed to its respective air space. Capillary loops dip around the angles of branches from the duct. At the lobular periphery, with the progressive disappearance of epithelium from the terminals, capillaries become exposed in these future air spaces.

Reticular fibers are now seen chiefly as condensed strands partially lining the spaces, or in the middle of thin walls between adjacent ones. With the continued thinning of the walls the latter arrangement becomes much more frequent so that with low magnification this part of the lung appears as a delicate lacy network. Collagenous fibers are thicker and denser around the larger tubes and blood vessels. Cartilage appears only in the largest bronchi.

At 290 mm., the length of the fetal pig at term, the number of epithelium-lined tubes in a section of a lobule is about the same as at 275 mm., but the tall columnar epithelium of the largest ones is more folded, and the columnar epithelium of the rest extends to the cuboidal epithelium of the terminal tubules. The latter have increased in diameter, and end still farther from the periphery of the much-enlarged lobule than at 275 mm. Cilia appear in the largest tubes of the lobule.

Mitoses are rare in the epithelium of a large bronchus, and are limited chiefly to that of the terminal bronchioles where they are seen occasionally. They appear fairly often in the end buds at all stages until they lose their epithelium, as most of them have at the present stage (290 mm.). Although

mitoses occur in the connective tissue at all stages, they are less frequent now.

In a given section two or more potential alveolar ducts arise from each terminal bronchiole. These ducts are more expanded and longer than the similar structures at 275 mm., and their many branches terminate in a closely packed mass of primordial air sacs which are so intermingled that ducts and sacs are separated only by thin connective tissue septa.

The large spaces separated by thin walls give the sectioned lung a lace-like appearance under low magnification. The large irregularly shaped 'septal' cells with vacuolated cytoplasm seen at 275 mm. are more prominent now, and more of them extend part way into the air spaces from the angles of septal walls and capillary loops.

Only occasionally does an epithelial cell appear on the walls of the alveolar ducts, and it is usually at the angle of protrusion of a capillary. A membrane cannot be seen over the endothelium of exposed capillaries. Nearly all the end buds are now potential respiratory spaces and are generally free of an epithelial lining except a few next to the pleura and the interlobular septa. In section these contain isolated epithelial cells or groups of two to four.

With the increase in number of thin connective tissue walls between the spaces, the capillaries become very prominent and exposed to the primordial air space. The epithelium in the terminal spaces loses its continuity where the capillaries penetrate the condensed reticular fibers and reach the base of the epithelium.

The reticular fibers lining adjacent primordial air spaces continue to approach one another and become confluent so that many more septa now have a single layer of condensed reticular fibers at least through part of their extent. These strands of reticular fibers occupy the centers of the septa while the capillaries either penetrate them, or lie on either side exposed to the future air space. Connective tissue cells are scattered among the reticular fibers and capillaries. It will be seen that in the postnatal stages this arrangement of the reticular fibers in the septa becomes the rule.

The condensed layer of reticular fibers is very heavy in the largest bronchi, but grades off through all the orders of branching until that at the base of the cuboidal cells in the terminal bronchiole at term may consist of but five or six fibers in section. These fibers become gradually reduced in number as they continue into the walls of all the potential air spaces, and finally assume the pattern described above. In this way the delicate strands of reticular fibers form a supporting framework for the capillaries and connective tissue cells of the finer air spaces.

Postnatal stages (6 and 18 hours)

In the postnatal stages the pulmonary veins were clamped and the heart allowed to beat a few strokes to fill the capillaries with blood before injecting the fixative at very low pressure by way of the trachea. The respiratory spaces were somewhat distended, but there was no tearing of tissues or extravasation of blood.

There is less folding of the epithelium in the large bronchi than at 290 mm., and many of the smaller bronchioles are lined with low columnar to cuboidal epithelium. There is no apparent change in number of tubes in a lobule. Mitoses appear fairly often in the epithelium of the smaller bronchioles, but rarely in the walls of the finer air spaces.

Presumably due in part to distension by the fixative, and in part to respiration, the septal and alveolar duct walls appear thinner and more openly lace-like than at 290 mm., and the lobules in section are much larger. Also, fewer air spaces appear in a given field (fig. 6). In most of the septa only a few scattered connective tissue cells appear in the rich network of capillaries. 'Septal cells' with vacuolated cytoplasm appear among the capillaries. Some of these show ameboid movement. The cytoplasm of these cells is more prominently vacuolated than in those at 290 mm.

There seem to be few if any epithelial cells or any sort of membrane in any alveolar ducts, although an occasional isolated cell (possibly of epithelial nature) may be present in the

lining of the air spaces next to the subpleural connective tissue (fig. 6). In places the faint ground substance of the connective tissue forms a thin film between the septal walls and the air spaces, but the capillary loops projecting into the air spaces appear bare.

The septal walls consist predominantly of a core of reticular fibers supporting networks of capillaries which wind back and forth through the septa and lie exposed for long stretches to the air spaces on either side.

SUMMARY AND DISCUSSION

In the early stages of development of the pig's lung there is a complete epithelial lining of the bronchial tubes. Later, the smallest tubes ramify and occupy progressively larger volumes of the lung. The epithelium becomes discontinuous so that the capillaries and connective tissue of these tubes are exposed directly to the lumen. Thus arise potential alveolar ducts which in turn will communicate with those areas in the postnatal lung where respiration will take place.

The early break in the epithelium divides the primordial air passages into three zones. With the reservation that it is impossible to homologize these structures exactly with those of the postnatal lung, these might be called 1) bronchi and bronchioles, lined with epithelium; 2) potential alveolar ducts, devoid of epithelium; 3) the end buds, lined with epithelium, which by growth and division irregularly extend the potential conducting and respiratory spaces. At term the epithelium in the end buds is partially or completely absent.

Probably the main factor in the exposure of the vascular connective tissue to the lumen of the duct system is the failure of the epithelium to keep pace with the very rapid extension of the branching terminals toward the lobular periphery. In this process the epithelium of the tubes stretches and breaks into fragments which lose their prominence as the primordial spaces expand.

The growth of the capillaries seems also to be a factor in the breaking and disappearance of the epithelium, as found

in the human fetus by Palmer ('35), for wherever in the future respiratory area a capillary pushes through the condensed reticular fibers and touches the base of the epithelial cells, these break apart, become irregular in shape and loosely attached, and their nuclei often become pycnotic. Although it seems clear that the embryo executes breathing movements for some time before birth, it has not been demonstrated that the aspirated amniotic liquid helps to distend the future respiratory spaces.

Though the epithelium is low cuboidal in places there is no evidence of overlapping phalanges as seen by Stewart ('23) in dogs and rabbits. Flint ('06) studied the development of the lung of the pig. He noted exposed capillaries and thought they were covered by a membrane much like non-nucleated plates. In my material non-nucleated plates were not seen at any stage even with the precarious silver nitrate technique. Silver often impregnates the capillary endothelium where exposed to the air space, and sometimes the outlines of connective tissue cells, as well as epithelial cells. Neither is the fine membrane over the capillaries in various mammals described by Seeman ('28) evident in the pig lung.

The final disposition of the reticular fibers in late fetal and postnatal stages is noteworthy because of their prominence in the septal walls, and because of their confinement chiefly to the center of the wall as a dense layer so that the capillaries lie on either side exposed to the air spaces, or pass through them from one space to the other. Of the scattered cells lying among the capillaries in the septal wall, many are associated closely with the reticular fibers, and probably have the potentiality of forming phagocytes. Frequently, where capillaries do not line the wall, reticular fibers and their ground substance, and connective tissue cells lie exposed to the air space.

The distinctive cell with irregular shape and vacuolated cytoplasm, possibly the septum cell of Lang ('25), needs to be traced in later postnatal stages. As it is followed from

275 mm. into the postnatal stages, it shows increase of vacuolization and depth of staining in the cytoplasm, and more actively ameboid forms. The fact that it is found within the septal wall as well as on its border partly projected into the air space indicates that it is not exclusively of entodermal origin.

CONCLUSIONS

1. In the fetal pig lung the walls of the future respiratory spaces are formed from the branching buds of the tubules which fill most of the room occupied in earlier stages by connective tissue.

2. As the end buds grow, the epithelium of the small tubules behind them becomes discontinuous, and gradually disappears, leaving potential ducts and spaces lined with capillaries and connective tissue.

3. The intertubular connective tissue of the early stages remains as the thin septa between the future air spaces.

4. The capillaries of the septa lie next to the future air space on either side of the walls and pass through them from one side to the other.

5. The reticular fibers continue from beneath the terminal bronchiolar epithelium into the centers of the septa between the potential respiratory spaces.

6. The fact that the epithelium begins to disappear in embryos of 200 mm. and is largely absent before term, indicates that the change from a series of epithelial-lined passages into those in which the epithelium is not apparent is obviously not due to the changes occurring at birth.

I wish gratefully to acknowledge my indebtedness to Dr. William Bloom, whose kind assistance made this work possible.

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DESCRIPTION OF PLATES

Figures 1 to 6. Semi-diagrammatic drawings, outlined with camera lucida at table level, from sections of fetal and postnatal pig lungs to show the fate of the epithelium in the developing respiratory spaces. Zeiss 8 mm. apochromatic objective and $\times 15$ ocular. A, alveolus-like pocket; B, branching tubules lined with epithelium; BRB, branching end bud; D, potential alveolar duct; D', alveolar duct; E, epithelium with black nuclei; EB, end bud, EC, exposed capillary, or connective tissue, where the epithelium is absent; EC', exposed capillary at end of epithelium of tubule; I, isolated epithelial cells; M, connective tissue, stippled; P, pleura; R, red blood cells and blood vessels in red; RC, strand of reticular fibers with capillaries on either side exposed to the air spaces; RT, condensed reticular fibers represented by a single black line at the base of the epithelium, and in the walls of the finer spaces; S, terminal expansions of the potential alveolar ducts; SB, side branch.

PLATE 1

EXPLANATION OF FIGURES

1 A 200-mm. stage showing branching tubules lined almost completely with epithelium, which is flattened in places, and interrupted occasionally. A capillary is exposed at EC. Note the relatively large amount of vascular connective tissue.

2 A 220-mm. stage. Epithelium-lined tubules, B. Potential alveolar ducts, D, with the epithelium partially absent appear between the tubules and the proliferating end buds.

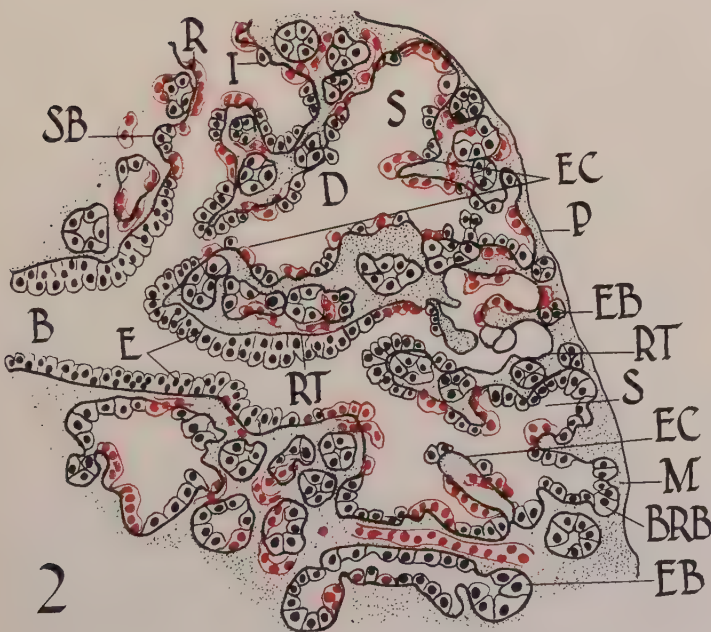
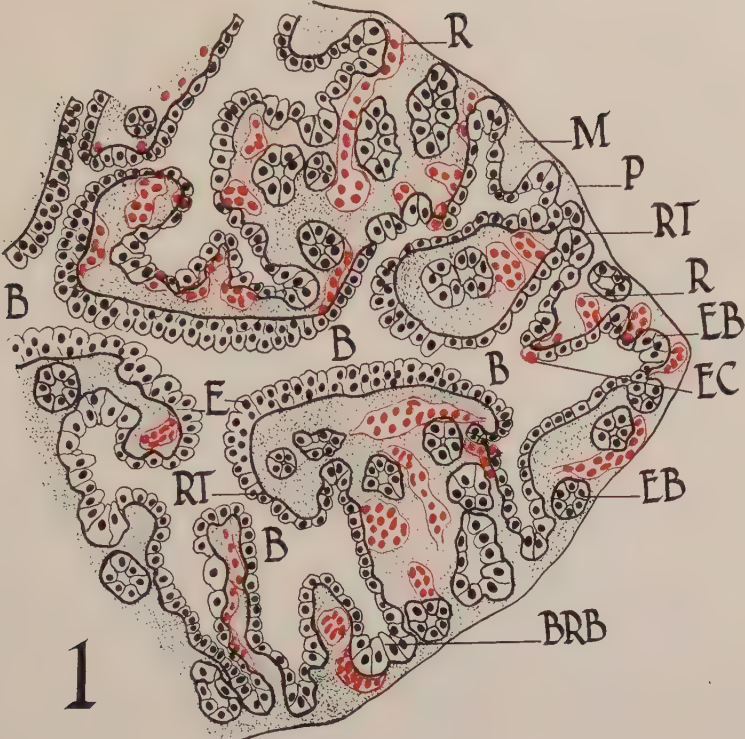


PLATE 2

EXPLANATION OF FIGURES

3 A 250-mm. stage. The epithelial-lined tube, B, branches and continues into several potential alveolar ducts, D, which lack a continuous epithelium. The terminal expansions of the ducts, S, are wider and more numerous than at 220 mm.

4 A 275-mm. stage. Little epithelium appears in the potential alveolar ducts, and most of that in the end buds is gone.

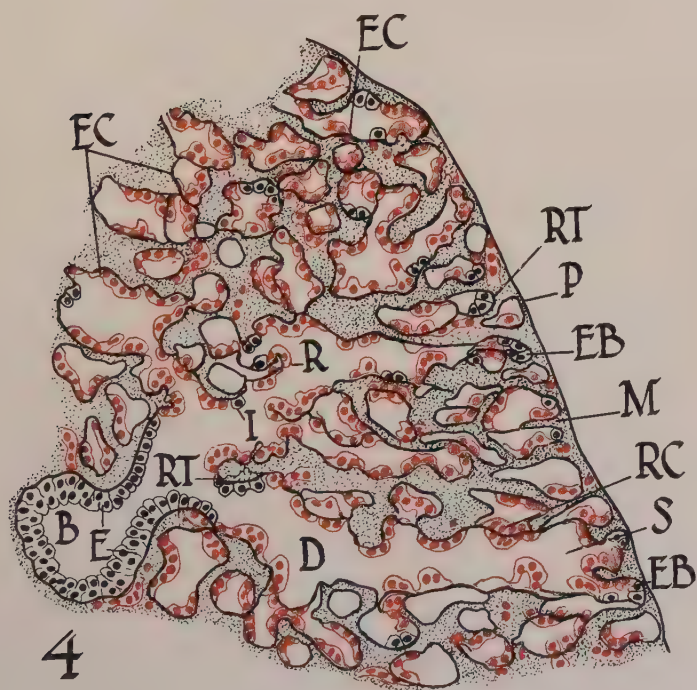
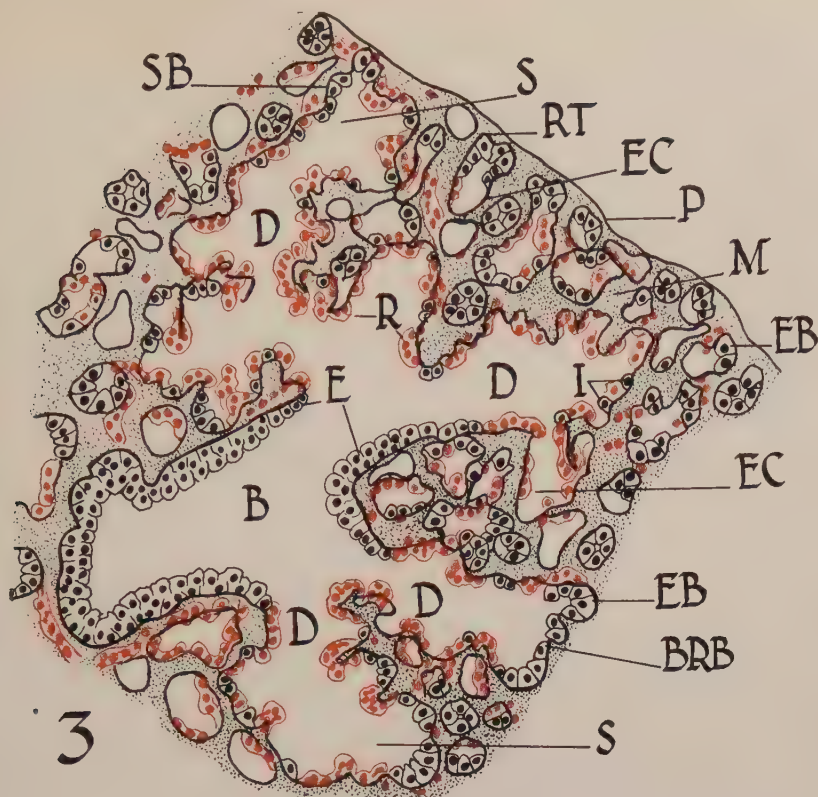


PLATE 3

EXPLANATION OF FIGURES

5 A 290 mm. (term). The alveolar ducts are further expanded so that the connective tissue between the spaces is much reduced. The epithelium is practically gone from the ducts and the capillaries and connective tissue are exposed to the air spaces. The epithelium is absent from most of the end buds, which are now potential respiratory spaces. The reticular fiber layers of adjacent spaces frequently become confluent as at RC.

6 Eighteen-hour postnatal stage. The respiratory spaces are much expanded by breathing and by the injected fixative, so that their number in the field is reduced. The capillaries lie exposed to the air spaces on either side of the reticular fibers in the septal walls. The connective tissue, M, is much reduced.

